PATENT
Docket No. 20296-20013.01

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Jennifer Taylor
Jennifer L. Taylor

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Chen et al.

Serial No.: 08/704,445

Filing Date: August 26, 1996

For: METHOD OF PREVENTING DEPLETION OF NON-AUTOLOGOUS HEMATOPOIETIC CELLS AND ANIMAL MODEL SYSTEMS FOR USE THEREOF

Examiner: S. Ziska

Group Art Unit: 1804

DECLARATION OF BEN CHEN PURSUANT TO 37 C.F.R. § 1.132

Box: AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Ben Chen, declare as follows:

1. I am an inventor of the above-referenced patent application, and am familiar with the contents thereof.

2. I currently reside at 2711 Parkside Drive, Fremont, California, 94536.

3. I have read the Office Action dated March 5, 1996 (the "March 5, 1996 Office Action"), issued in connection with the parent application, U.S. Serial No. 08/169,293, filed December 17, 1993, upon which the above-referenced file wrapper continuation application

under 37 C.F.R. § 1.62 was filed. I understand that claims 1-31 stand rejected in the parent application and in the subject application. I understand that the Office has rejected claims 1 to 17 and 19 to 23 for allegedly being obvious in view of Aldrovandi et al. (Nature 363:732-736 (1993); "Aldrovandi") taken with Pinto et al. (J. Leukocyte Biol. 49:579-586 (1991); "Pinto"), that claim 18 has been rejected under 35 U.S.C. § 103 as allegedly unpatentable over Aldrovandi and Pinto in view of Bernstein et al. (J. Clin. Invest. 88:540-545 (1991); "Bernstein"), that claims 24-30 have been rejected under 35 U.S.C. § 103 as allegedly unpatentable over Berenson et al. (Blood 77:1717-1722 (1991); "Berenson") and Baum et al. (Proc. Natl. Acad. Sci. 89:2804-2808 (1992); "Baum") taken with Pinto and that claim 31 has been rejected under 35 U.S.C. § 103 as allegedly unpatentable over Baum taken with Pinto.

4. This declaration is submitted in traversal of the rejection of the claims under 35 U.S.C. § 103 and more specifically, to traverse the Office's position that "... it is well known in the art that macrophages are involved in the immune response regarding the rejection of transplanted or injected tissues... " (page 4 of the March 5, 1996 Office Action). The Office concluded that the claimed invention would have been obvious to one of ordinary skill in the art at the time the invention was made based on the teachings of Pinto that dichloromethylene diphosphonate (DMDP) "decreases the number of endogenous macrophages and the results of a decreased number of macrophages would be numerous in view of the major role of macrophages in maintaining the immune response." (bridging paragraph of pages 4-5 of the March 5, 1996 Office Action).

5. Attached hereto are Exhibit A, Exhibit B and Exhibit C. Exhibit A is a publication entitled "Allograft and xenograft rejection in C3H/SCID mice. A new model for the study of non-T cell graft rejection mechanisms." Marcus et al., Transplantation 61:777-783 (1996) ("Marcus"). Exhibit B is a publication entitled "Engraftment of human lymphoid cells into newborn SCID mice leads to graft-versus-host disease." Pflumio et al., International Immunology 5:1509-1522 (1993) ("Pflumio"). Exhibit C is a publication entitled "High level functional engraftment of severe combined immunodeficient mice with human peripheral blood lymphocytes following pretreatment with radiation and anti-asialo G_{M1}." Shpitz et al., J. Immunol. Methods 169:1-15 (1994) ("Shpitz").

6. In Marcus, the authors address the problem of low engraftment of allogeneic and xenogeneic cells in SCID mice. The publication describes the transfer of allogeneic and xenogeneic cells into two strains of SCID mice -- C3H/SCID and C.B-17/SCID. Allogeneic C.B-17/SCID bone marrow cells were engrafted poorly compared with syngeneic C3H/SCID when transplanted into C3H/SCID recipients, whereas cells of both strains were equally well engrafted into C.B-17/SCID mice. (page 779, column 1, Table 2). One month after transplantation of C3H/SCID and C.B-17/SCID mice with human 70 x 10^6 peripheral blood lymphocytes, the total human Ig was 199.8 ± 46.2 μg/ml in the C3H/SCID mice compared with 1360 ± 341.1 μg/ml in the C.B-17/SCID mice group. (page 781, column 2, paragraph 2, lines 2-7). C.B-17/SCID mice were much more permissive for out-growth of human Burkitt lymphoma (Raji) cells. (bridging paragraph of pages 780 and 781). The resistance to human Raji cells exhibited by C3H/SCID mice could be adoptively transferred by infusion of C3H/SCID splenocytes into C.B-17/SCID mice. (page 781, column 2, paragraph 3 and page 781, column 1, Figure 3). The authors conclude that the marked immunoreactivity against foreign grafts is mediated by non-T non-B mechanisms, (page 782, column 1, paragraph 1, lines 7-9) and point toward NK cells as the cells possibly responsible for low engraftment levels (page 782, column 1, lines 12-17).

7. The results reported in Marcus show that, at the time of publication of the reference, it was unclear precisely which cell population(s) or other factor(s) are responsible for rejection of non-autologous cells in engrafted SCID mice. The authors suggest that, since SCID mice lack $CD4^+$ and $CD8^+$ T cell function, these mice provide relevant models for studies of non-T cell, non-B cell mechanisms of allograft or xenograft rejection. (page 782, bridging paragraph of columns 1 and 2). The authors suggest that another population, perhaps NK cells, are the mediators of the rejection in SCID mice, but were unable to point conclusively toward any one cell type. (page 782, column 1, paragraph 3, lines 6-12). Therefore, it would not have been obvious to one of ordinary skill in the art to deplete macrophages in order to avoid rejection of non-autologous cells by SCID mice engrafted with human hematopoietic cells.

8. In Pflumio, the authors of the publication address the problem of low engraftment of murine tissues with functional human T and B cells when adult SCID mice are engrafted with human lymphoid tissue. Newborn SCID mice were injected with human bone marrow and

peripheral blood leukocytes. Newborn SCID mice were specifically chosen as recipients for human cell engraftment because natural killer cell activity and other immune functions do not develop until several weeks after birth. (page 1510, column 1, paragraph 2). The extent of human cell engraftment was measured by probing blots of EcoRI-cut and electrophoresed DNA for the presence of human α-satellite DNA. The functionality of engrafted cells was assessed by measuring the concentration of human immunoglobulin in the serum and monitoring the frequency of symptoms resembling graft-versus-host disease (GVHD). The results showed that human cells spread to many organs. (page 1511, column 2, paragraph 4, lines 10-13; page 1512, Figure 1; page 1513, column 1, Figure 2). In addition, many mice exhibited GVHD-like symptoms: these mice became sick within 2-4 weeks following transplantation, and histological analyses of organs with human lymphoid infiltrates revealed patterns of tissue destruction consistent with GVHD. (pages 1518 and 1519, Figure 7; page 1517, column 1, lines 6-9 and 18-26). This fact, coupled with the presence of human IgG and IgM antibodies (page 1516, column 1, Figure 5; page 1516, column 2, paragraph 1), indicated that the engrafted human cells retained some immune functions.

9. The results reported in Pflumio show that, contrary to the Office's position that it would have been obvious to one of ordinary skill that depleting macrophages would prevent rejection of non-autologous cells in engrafted SCID mice. The results also show that contrary to the Office's position, the population(s) of cells responsible for such rejection was in fact unknown in 1993. The authors reported that, compared with the literature on the use of adult SCID mice as recipients, the transplanted cell dose using newborn SCID mice was much lower and the human cell infiltration of the mouse hematopoietic and non-hematopoietic organs was more extensive. (page 1510, column 2, paragraph 1, lines 4-8; page 1518, column 1, paragraph 1, lines 1-12). Since newborn SCID mice lack the NK cell function of adult SCID mice, the authors suggested that NK cells were perhaps the mediators of rejection in adult SCID mice, but did not draw any firm conclusions as to the identity of the cell type involved. (page 1510, column 1, paragraph 2, lines 2-8; page 1518, column 2, lines 3-11).

10. In Sphitz, the authors address the problem of low and variable short term human peripheral blood lymphocyte (PBL) engraftment in SCID mice, noting that, in addition to showing low levels of engraftment, human T cell engraftment in the lymphoid organs of hu-

PBL-SCID mice is significantly limited during the early post-reconstitution period, as the human PBLs appear to remain predominantly in the peritoneal cavity. (page 2, column 1, paragraph 1, lines 17-29). To overcome these problems, various treatment protocols were pursued. The mean level of $CD3^+$ cells in the spleen was < 5% in untreated SCID mice injected with human PBLs. Depletion of mouse NK cells by pre-treatment with anti-asialo G_{M1} rabbit polyclonal antibody resulted in a marginal improvement of short term reconstitution with human $CD3^+$ cells, while preirradiation with 3 Gy improved reconstitution to over 16% $CD3^+$ cells on days 12-14 following engraftment. However, when a combination of pretreatment with anti-asialo G_{M1} plus irradiation was pursued, the mean percentage of human $CD3^+$ cells in the spleen increased to 40% within 2 weeks following injection of PBLs. (page 5, Figure 1; bridging paragraph of pages 4 and 6). The human immune cells in these mice were shown to be functional by the *in vivo* demonstration of an appropriate secondary immune response to the injection of tetanus toxoid (page 9, bridging paragraph of columns 1 and 2, and bridging paragraph of pages 9 and 10; page 9, column 2, Figure 5) and by an *in vitro* proliferative response to phytohemagglutinin. (page 9, column 1, Table 4). The authors postulated that the role of anti-asialo G_{M1} treatment in the successful engraftment of human PBLs was reduction in endogenous NK activity in SCID mice. (page 13, column 1, paragraph 1, lines 6-10). The mechanism by which preirradiation of SCID mice improves engraftment with human PBLs was stated to be unknown. (page 13, column 1, paragraph 1, lines 4-6).

11. The results reported in Shpitz show that, contrary to the Examiner's assertion that it would have been obvious to one of ordinary skill to deplete macrophages in order to prevent depletion of non-autologous cells in engrafted SCID mice, the cell type responsible for the poor engraftment was unknown, even at the time of publication of Shpitz. The authors observed that pretreatment with anti-asialo G_{M1} plus γ-irradiation resulted in increased numbers of functional human $CD3^+$ cells in the spleen, and attributed the increase to a reduction in the NK cell population. (page 13, column 1, paragraph 1, lines 1-10).

12. Taken together, the teachings of Marcus, Pflumio and Shpitz show that, at the time the application describing the present invention was filed, the teachings of the art did not identify which cell population(s) or which other factor(s) are the mediators of non-autologous

cell depletion in engrafted SCID mice. Accordingly, it would not have been obvious to try reduction of the macrophage population as a means of obviating such depletion.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

12/17/96 Benjamin Chen

Date Ben Chen